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Yellow Dwarf a Virus Disease of Onions, and Its Control

By W. J. HENDERSON

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
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SUMMARY

Yellow dwarf of onions is a transmissible virus disease, of the mosaic group. The virus overwinters in the infected onion bulbs and in volunteer onion plants that live over winter in the fields. It probably is neither seed nor soil borne.

Yellow dwarf symptoms are characterized by yellowing and crinkling of the leaves, which become more or less flat and droop over in the advanced stages. Flower stalks of infected mother onion plants become yellow, twist, and curl and are shorter than normal plants. Masking of yellow dwarf symptoms occurs quite commonly in infected onion plants, and these plants are a source of inoculum for infecting healthy plants; bulbs from the plants masking symptoms when regrown produce infected plants that show disease symptoms.

Plants grown from infected onion sets and those that become infected early in the growing season produce under-developed bulbs of little commercial value. Onion plants having masked infection throughout their growth period produce apparently normal plants and yields. Infected mother onion plants produce normal seed, but the seed yield is about 30 percent less than from healthy plants.

Yellow Dwarf is transmissible by artificial inoculation and insect vectors. By artificial inoculations the incubation period is usually about 10 days. Onion bulbs hypodermically inoculated with juice extracted from the fleshy and dry scale leaves of infected onion bulbs failed to show infection. Inoculations made during retarded growth periods either fail to infect the plants or the symptoms are not manifested during the current growth period. Signs of the disease appear when the bulbs of such infected plants are regrown. The virus extracted from yellow dwarf infected onion leaves that mask the symptoms is infective.

Yellow dwarf virus was transmitted under controlled conditions by the bean aphid (*Aphis rumicis* Linn.), corn leaf aphid (*Aphis maidis* Fitch.), and the apple grain aphid (*Rhopalosiphum prunifoliae* Fitch.).

The yellow dwarf virus was inactivated when the viriferous juice was stored in vitro at 29° C. for a period of 112 hours.

When infected onion leaves were stored in the open, at 29° C. for 100 hours, juice extracted from the leaves was non-infective.

The infectivity of the virus was only slightly retarded when viriferous juice was heated 10 minutes at 70° C. At 75° C. for 10 minutes, 55 percent of the original virulence of the virus was lost. At 80° C. for 10 minutes, the virus was inactivated. With 10-minute exposures the critical thermal point for the yellow dwarf virus lies between 75 and 80° C.

Exposures as low as -14° C. for 6 hours failed to inactivate yellow dwarf virus.

Inoculations with dilutions of 1-10,240 and above failed to infect any of the onion plants.

Yellow dwarf virus inoculated into Chinese sacred lily (*Narcissus tazetta* L.) bulbs, Jonquil (*Narcissus jonquilla* L.) bulbs and shallots (*Allium sativum* L.) visibly infected 60, 30 and 90 percent of the plants, respectively.

Inoculations with other plant viruses, including mosaic of Chinese sacred lily (*Narcissus tazetta* L.) and Jonquil (*Narcissus jonquilla* L.) failed to infect onion plants.

Only one (Riverside Sweet Spanish) of thirty-six onion varieties tested seemed to be markedly resistant.

The combined effect of indexing all growing stocks of onion bulbs, producing the planting stock of bulbs in areas free from yellow dwarf and roguing out the infected volunteer onions in the fields reduced the percentage of yellow dwarf infection in the district from 40 percent in 1928 to a trace in 1933 and 1934.

Yellow Dwarf, a Virus Disease of Onions, and Its Control

BY W. J. HENDERSON

A virus disease of onions, now known as yellow dwarf, was first found in Iowa in June, 1927, in the Pleasant Valley onion growing district. At that time, 50 to 60 percent of the plants in two or three fields of table onions grown from sets showed yellow dwarf. During the following season, 1928, an epidemic occurred in which 35 to 40 percent of the table onions in the entire district became diseased, and in certain fields 75 to 95 percent. In the same season seed crops, which are grown from mother-bulbs, were 85 to 95 percent diseased so that a very light crop of poor quality seed was produced.

The heavy losses sustained and their probable profound effect upon this old intensive onion district, if they continued, caused a demand for a thorough study of the disease. The study was initiated in June, 1928, with work centered in a field laboratory in the onion district during the growing season and continued in the phytopathological laboratories at Ames during the remainder of the year. The unknown nature of the disease made imperative a study of the symptoms, causal agent, host relations and control measures. Observations and data are recorded in the following pages in the order enumerated above.

NAME

When this virus disease was first discovered in the Pleasant Valley district its cause was not known. The most characteristic symptoms were a yellowing, crinkling and dwarfing of the foliage and flower stalks. These symptoms suggested the name yellow dwarf for this malady. Subsequently, it was learned that Dr. N. J. Giddings had observed a mosaiclke onion disease in West Virginia, but he was not able in a limited study to establish its causation. Now that it has been shown that the yellow dwarf virus is readily transmissible artificially and also because the very early symptoms show a mosaic pattern, it seems evident that yellow dwarf falls in the mosaic group rather than the yel-

¹ These studies were made under project No. 91 of the Iowa Agricultural Experiment Station, "A study of the yellow dwarf and other onion diseases of Iowa." The author is greatly indebted to Dr. I. E. Melhus and Dr. C. S. Reddy for their advice and helpful suggestions during the progress of the work and in preparation of this manuscript. The author also takes pleasure in acknowledging the very generous assistance extended by Pleasant Valley onion growers. Special mention is due Mr. Russell Rice and Mr. Albert Schutter in that they loaned equipment, housed seed stocks, and often assisted with routine practices incident to growing experimental plots.

lows group, In this publication, however, the original common name, yellow dwarf, will be used because of its descriptive value and general use by growers.

SYMPTOMS

YELLOWING

Symptoms of the yellow dwarf disease are easily detected when they are manifested, but masking of symptoms by infected onion plants is common. The initial manifestation of symptoms, in plants grown from naturally infected onion bulbs, is a series of short yellow streaks which appear at the base of the first leaf emerging through the neck of the bulb. Onion plants inoculated in the leaves either by the needle method or by insect vectors, show these first symptoms only at the base of the leaves emerging after the inoculation. All leaves, in general, that emerge after the appearance of the first symptoms, show signs of the disease and those previously developed remain apparently healthy. Under conditions favorable for development of yellow dwarf disease the leaves showing the short yellow streaks at the base become yellow throughout and also crinkled and somewhat flat. In this condition the leaves lop over and present the abnormal appearance shown in figs. 1 and 2.



Fig. 1. Yellow dwarf disease on four Yellow Bottleneck onion plants. Healthy onions of same variety in center.

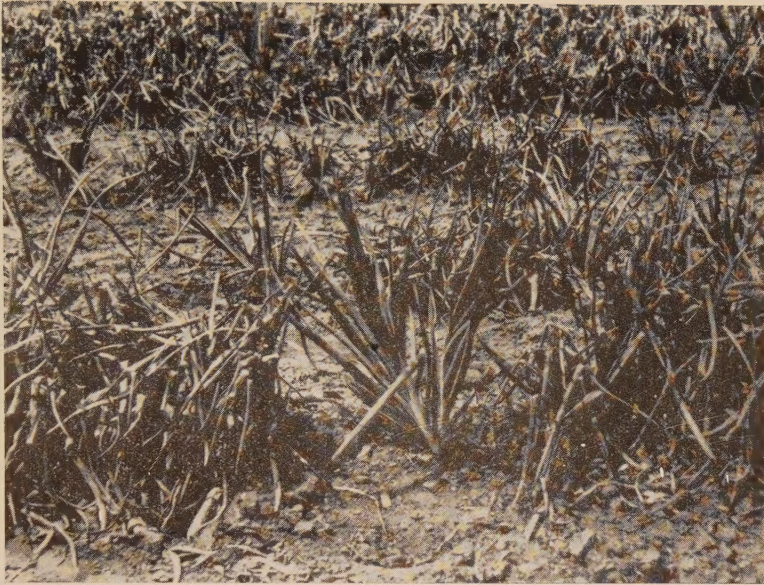


Fig. 2. Diseased Yellow Bottleneck onions surrounding one healthy plant of the same variety in a field at Pleasant Valley, Iowa, 1928.

Flower stalks of infected plants producing seed show yellow streaks extending upward from the base. Later the streaks coalesce, the stalk becomes yellow throughout and twists and curls in a characteristic manner (figs. 3 and 4).

DWARFING

The yellow, crinkled and lopped-over condition of table onion plant leaves and the twisted and curled flower stems of infected plants producing seed, give the diseased plants a decidedly dwarfed appearance (figs. 1, 2, 3 and 4). Bulbs of onion plants produced from infected sets are under-developed and of little commercial value, although they are usually well shaped. Bulbs from onion plants with only the last few leaves showing symptoms, or from plants with completely masked symptoms, are usually normal in development and appearance. Floral clusters of infected mother-onion plants are smaller and have fewer flowers than normal plants (figs. 3 and 4).

MASKING OF SYMPTOMS

Although manifested symptoms of yellow dwarf are easily detected, a large percentage of infected onion plants either completely masked the symptoms or only a few of the later developed leaves showed signs of the disease. The former group cannot be determined by observation and the latter group may be

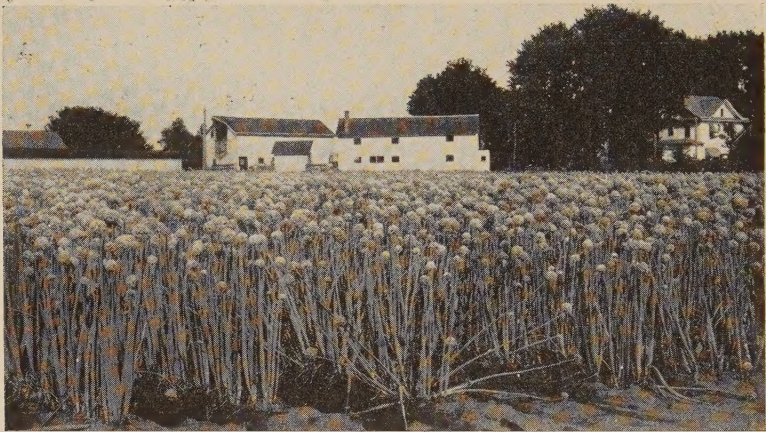


Fig. 3. A crop of healthy Yellow Bottleneck onion plants at Pleasant Valley, Iowa, 1931.

easily overlooked. Early in the season of 1928, it was found that the yellow dwarf virus was carried in the onion bulbs. To study masking of symptoms of yellow dwarf disease on onions a series of experiments was conducted, the data from which are presented in table 1. July 1, 1928, 200 Yellow Bottleneck onion sets and 200 Red Globe table onion bulbs from apparently healthy plants were collected at random from each field of R. M. Rice, F. H. Schutter and Albert Schutter. These bulbs were planted in a greenhouse bed Sept. 19, 1928. The percentages of plants that showed signs of yellow dwarf were determined after 35 days (Exp. 1, table 1).

June 26, 1928, 200 apparently healthy plants were located in a field of Red Globe table onions on the C. Kramback farm, in which 95 percent of the plants showed symptoms of yellow dwarf. Two weeks later 96 of these plants which were apparently healthy when observed June 26 showed signs of the

TABLE 1. DATA FROM FOUR EXPERIMENTS IN WHICH YELLOW DWARF SYMPTOMS WERE MASKED IN THE FIRST GROWTH PERIOD BUT NOT IN THE SECOND.

Exp. No.	Sources of material	First Growth period		Second growth period	
		Number plants	Percentage infection	Number plants	Percentage infection
1	Rice sets	200	none infected	200	30.0
	F. H. Schutter sets	200		200	42.0
	A. Schutter sets	200		200	28.0
	Rice commercial bulbs	200		200	48.0
	F. H. Schutter commercial bulbs	200		200	32.0
	A. Schutter commercial bulbs	200		200	44.0
	Kramback commercial bulbs	104		50	98.0
2	Hypodermically inoculated sets	21		21	33.3
4	Schutter shaded sets	1624		200	14.0

disease on the last few leaves that developed. The other leaves gave no manifestations of symptoms. The bulbs of the remaining 104 apparently healthy plants that had been labelled were harvested. By April 5, 1929, 54 of these bulbs had been destroyed by soft rot; the remaining 50 bulbs were planted. May 6, 1929, the percentage of plants that showed yellow dwarf symptoms was taken (Exp. 2, table 1).

Twenty-one healthy Yellow Globe onion bulbs were hypodermically inoculated April 5, 1930, with juice of yellow dwarf infected onions, and sterile water was injected into 20 others to serve as checks. The bulbs were planted in 6-inch pots of soil in the greenhouse and kept separate from other onion plants. These plants were observed throughout the summer, but no symptoms appeared. July 28, 1930, the bulbs were harvested and Sept. 10, 1930, were planted a second time. Thirty days later, 33 percent of the plants which had been inoculated expressed yellow dwarf symptoms and the uninoculated checks remained healthy (Exp. 3, table 1).

May 10, 1930, a lath frame was constructed over a square rod of Red Globe onion seedlings on the Albert Schutter farm. The plants inside the frame did not grow normally, because of the shading, and none of them showed any signs of yellow dwarf. Nine percent of the plants of the field crop onions growing adjacent to the frame showed yellow dwarf symptoms. July 8, 1930, the bulbs inside the frame were harvested and Oct. 16, 1930, a random sample of 200 was planted in the greenhouse. Forty days later, 14 percent of the plants manifested symptom of the disease (Exp. 4, table 1).

Data from experiment 1 recorded in table 1 show that when Yellow Bottleneck sets from apparently healthy plants col-



Fig. 4. Diseased Yellow Bottleneck onion plants at Pleasant Valley, Iowa, 1928. Ninety-five percent of the plants were diseased.

lected in three fields were regrown in the greenhouse, 30.0, 42.0 and 28.0 percent, respectively, of the plants expressed yellow dwarf symptoms and bulbs from apparently healthy Yellow Bottleneck table onions, collected in three fields and treated in the same manner showed 48.0, 32.0 and 44.0 percent visible infection, respectively. Data from experiment 2 in table 1 show that 98 percent of the plants in a similar experiment masked the symptoms during the first growth period but expressed them during the second. Data from experiment 3, table 1, show that artificially inoculated bulbs masked the symptoms during the first growth period and 33.3 percent expressed symptoms during the second. Data from experiment 4, table 1, show that shading by the lath caused complete masking of symptoms of yellow dwarf of onions.

A very probable reason why the infected table onion plants failed to manifest disease symptoms during the growing season was because they became infected late in the season when the plants were approaching maturity, and growth was retarded. Similarly, onion seedlings for production of sets seldom show yellow dwarf symptoms regardless of the time they become inoculated with the virus. This is because they are planted so thickly in rows that growth is very much retarded. Onion plants that have been hypodermically inoculated in the bulbs often fail to express symptoms during the current growth period, because of faulty inoculation technique. Inoculum injected into the storage tissue adjacent to the growing point of the bulb is slow—or fails—to reach the meristematic tissue at the time the plant is making vigorous growth, thus the symptoms of the disease are not manifested until the bulb has been regrown (the next season). In experiment 4, table 1, the shaded plants were growing under adverse conditions which retarded growth and caused masking of disease symptoms. The relation of the condition of the host at inoculation time to the effects produced will be discussed later.

PREVALENCE AND DISTRIBUTION OF YELLOW DWARF OF ONIONS

In Iowa yellow dwarf disease was found in the Pleasant Valley onion district which comprises about 1,000 acres in an irregular strip adjacent to the Mississippi river. Approximately 90 percent of the entire onion crop of this district is produced in the river bottoms extending between Pleasant Valley and Bettendorf. The remainder of the crop is grown in the region back of the river bluffs near Davenport, Bettendorf, Pleasant Valley, LeClaire and Princeton, Iowa.

In 1928 the center of infection was located in the vicinity of Pleasant Valley, where 95 percent of the onion plants were visibly infected. The percentage of disease in onion fields ad-

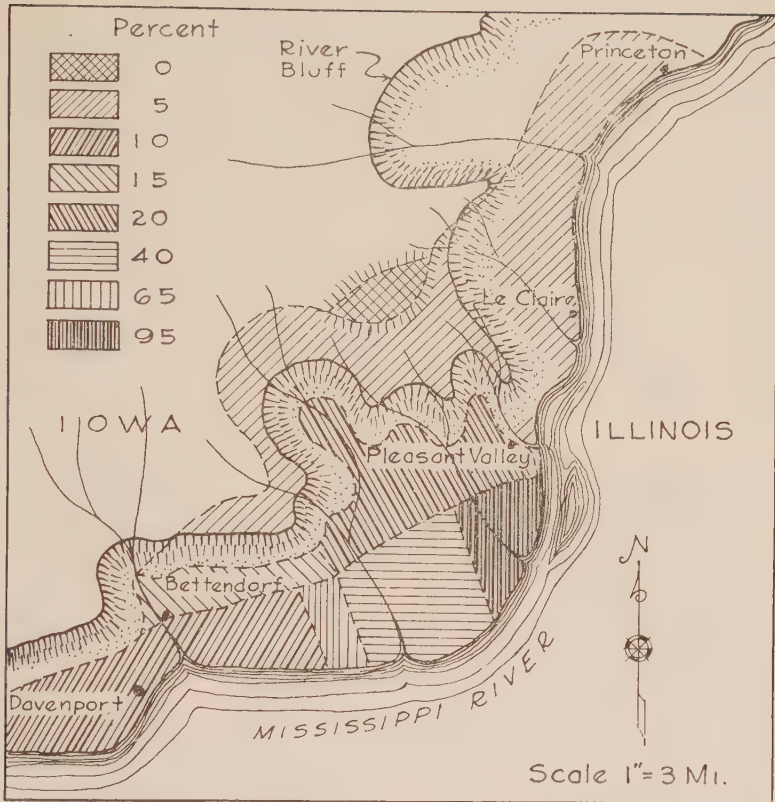


Fig. 5. Onion growing areas of eastern Iowa showing distribution of yellow dwarf in 1929.

adjacent to the river decreased from Pleasant Valley to Bettendorf so that this area could be divided into sections in which approximately 95, 65, 40 and 10 percent of the plants, respectively, showed yellow dwarf symptoms. The bottom land between this area and the river bluffs produced onion crops that were 15 to 20 percent visibly infected. In the region back of the river bluffs and in the fields in the vicinity of LeClaire and Princeton, the crops were about 5 percent visibly infected with yellow dwarf, except a small area near LeClaire, where the crop was apparently free from infection (fig. 5).

In addition to Iowa, yellow dwarf of onions has been found in West Virginia, California and Minnesota. Giddings (5) reported a serious onion disease occurring in the southern part of West Virginia. However, he did not prove the virus nature of the disease, but in correspondence with Dr. I. E. Melhus in 1929 he states, "Some years ago we found a disease which we

designated as onion mosaic. It apparently was carried only in the vegetative parts, or sets, and at that time we found it only in what are known as multiplier or potato onions. The disease was extremely prevalent in the south central part of the state and they urged us to do something to help them. We made a large number of attempted isolations, but could obtain nothing . . . , and from the general symptoms concluded that the disease was a mosaic."

Bremer (2), 1929, reported a disease of onions in Germany characterized by drooping, crinkling and yellow streaking of the leaves. He states, "During the summer of 1929, the disease was reported from various parts of Germany as causing 60 to 95 percent infection, especially in the second year stands." He called attention to the similarity between these symptoms and those of yellow dwarf in Iowa.

INOCULATION TESTS

In 1929, Dr. N. J. Giddings sent specimens of infected onion bulbs from West Virginia to Ames where they were grown in the greenhouse and showed typical symptoms of yellow dwarf. In the autumn of 1929, Dr. L. D. Leach collected specimens of yellow dwarf infected onion plants in California and sent them to Ames. Inoculation tests proved that the disease was yellow dwarf. In 1931, Dr. I. E. Melhus collected specimens of yellow dwarf diseased plants in Minnesota. Healthy onion bulbs, inoculated with juice extracted from these plants and grown for a short time showed typical symptoms of yellow dwarf. The results of hypodermically inoculating healthy onion bulbs with juice extracted from each of the specimens received are given in table 2.

Table 2 shows that a large percentage of the plants in each of the tests produced typical symptoms of yellow dwarf when healthy onion bulbs were hypodermically inoculated with juice extracted from infected onion plants collected in different

TABLE 2. RESULTS OF INOCULATING HEALTHY ONION BULBS IN THE GROWING POINTS WITH JUICE FROM SUSPECTED YELLOW DWARF DISEASED ONION PLANTS OBTAINED FROM DIFFERENT STATES.

Source of virus	Date of inoculation	Number of bulbs		Percentage of yellow dwarf	
		Inoculated	Checks	Inoculated	Checks
Iowa	June 10, 1929	100	100	85.0	0.0
West Virginia	Oct. 6, 1928	30	30	63.0	
California	Sept. 22, 1929	50	50	88.0	
Minnesota	Sept. 16, 1931	50	40	70.0	

states. It is evident, therefore, that Dr. Giddings in 1916 was dealing with the same disease in West Virginia and that it was present in California in 1929 and in Minnesota in 1931.

NATURAL SPREAD OF YELLOW DWARF VIRUS

An opportunity for observation of the natural spread of the disease occurred in June, 1929, when a field of Red Globe onions grown from seed was located adjacent to a field of Yellow Bottleneck onions grown from sets. Thirty percent of the Yellow Bottleneck plants showed symptoms of yellow dwarf early in the season, and the first appearance of the disease in the Red Globe field was definitely adjacent to this diseased field. Within 4 weeks there was a progressive spread over the Red Globe field so that at harvest 65 percent of the plants showed symptoms of yellow dwarf.

July 25, 1928, after harvest, 2,000 healthy onion bulbs were planted in a field where the previous crop had shown 65 percent infection. The bulbs were dormant when planted and did not start growth until Sept. 20. At the same time hundreds of infected volunteer onion plants were growing in this field in the vicinity of the plot. By Oct. 16, when the plants in the experimental plots were 8-19 inches high, 30 percent showed yellow dwarf symptoms. Results of this test clearly indicated that natural transmission of the yellow dwarf virus could occur late in the season.

The following year, 1929, Red Globe onion seed again was planted in the field referred to in the preceding paragraph. It was bordered on the north by a bluegrass pasture, on the south by a weed-covered railroad right-of-way, on the east by a crop of Yellow Bottleneck onions from sets (in which 20 percent of the plants were infected) and on the west by a field of Red Globe onions grown from seed. The field was divided into 19 sections, 16 of equal size and three small ones. From April 20, when the onion seedlings were 2-3 inches in height—3 weeks after the plants grown from infected sets had shown evidence of yellow dwarf—until harvest time, July 7, the percentage of plants manifesting symptoms in each section was recorded at weekly intervals. Fig. 6 shows the method of sectioning the field and its bordering crops. Percentages of yellow dwarf found in plants of the various sections of this field on different dates are given in table 3.

Experiments in 1929 showed that yellow dwarf was not seed-borne. Any yellow dwarf in onion plants grown from seed, therefore, was due to inoculum from some other source. Data given in table 3 and fig. 6 show that the first evidence of yellow dwarf symptoms in this field of Red Globe onions appeared June 2, in sections 1 and 2. On June 9, the number of plants

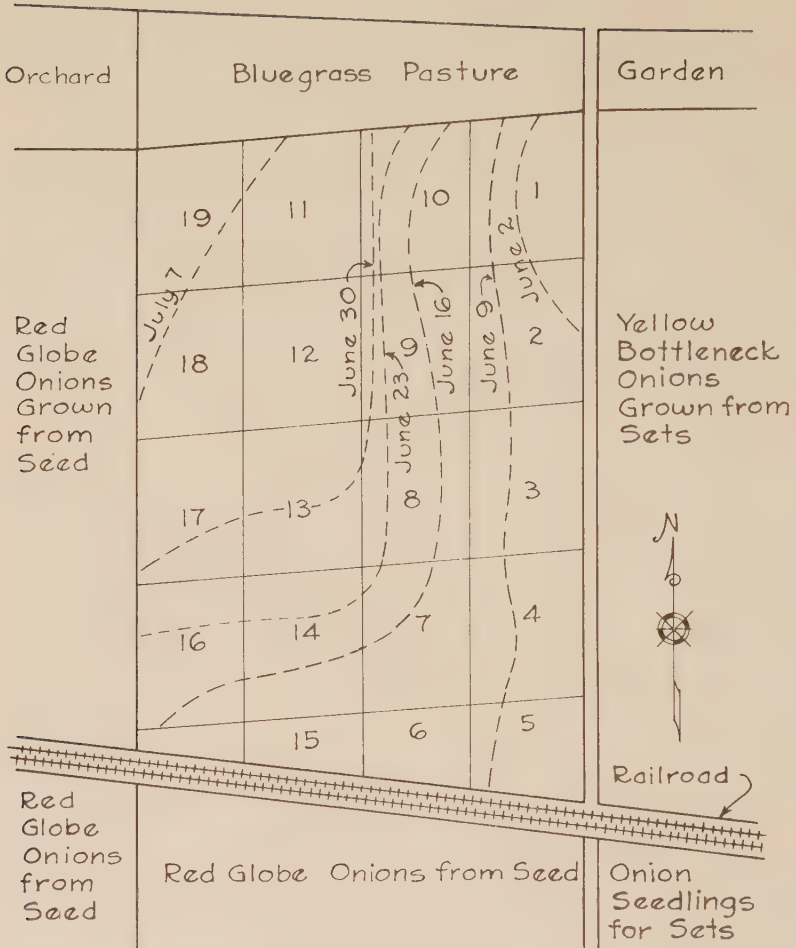


Fig. 6. Location of numbered field sections referred to in table 3. Broken lines show spread of yellow dwarf virus in a field of Red Globe table onions from seed, Pleasant Valley, Iowa, 1929.

showing yellow dwarf had increased in sections 1 and 2 and had appeared in sections 3, 4 and 5. Sections 3, 4 and 5 were bordered on the east by a crop of Yellow Bottleneck onions grown from sets, where 20 percent of the plants had shown yellow dwarf symptoms for the preceding 3 weeks. June 16 there was a marked increase in percentages of yellow dwarf in those sections where it had previously been found, and the disease appeared in sections 6, 7, 8, 9, 10, 14, 15 and 16. June 23 the disease had spread and increased in all the above mentioned sections, but none had appeared in the others. June 30 large increases in percentages of infection occurred in all sections

where yellow dwarf had been previously found, and the disease appeared in sections 13 and 17 for the first time. The plants in sections 11, 12, 18, and 19, located in the northwest quarter of the field, did not show yellow dwarf symptoms until July 7. On that date from 2 to 65 percent of the plants in the different sections of the field showed symptoms of yellow dwarf. Those sections adjacent to the Yellow Bottleneck onion crop—showing yellow dwarf—and the railroad tracks were most heavily diseased, which indicated that the agency of natural transmission came from the Yellow Bottleneck onions.

Yellow dwarf symptoms continued to appear in plants of the different sections between June 2-July 7. Because of the disease's incubation period, the insects responsible for natural transmission of yellow dwarf virus to these plants necessarily would have been present in the fields during the period May 18-June 25.

In 1930 and 1931, although the percentage of yellow dwarf throughout the entire district had been greatly reduced, there was similar evidence of natural transmission in the onion crops grown from seed.

Melhus, Reddy, Henderson and Vestal (9) showed that yellow dwarf is a virus disease. Further studies of artificial methods of transmission and spread of yellow dwarf under field conditions have been made to ascertain its infectivity and possible means of dissemination.

TABLE 3. PERCENTAGES OF DISEASED PLANTS FOUND IN DIFFERENT SECTIONS* OF AN ONION FIELD PLANTED WITH RED GLOBE SEED AND READ AT WEEKLY INTERVALS.**

Section	June					July
	2	9	16	23	30	7
1	1.0	5.0	8.0	15.0	24.0	35.0
2	1.0	5.0	6.0	12.0	20.0	30.0
3		2.0	4.0	9.0	19.0	30.0
4		5.0	8.0	12.0	20.0	50.0
5		4.0	9.0	16.0	36.0	65.0
6			10.0	18.0	34.0	65.0
7			5.0	15.0	20.0	50.0
8			2.0	5.0	15.0	35.0
9			2.0	6.0	14.0	30.0
10			1.0	4.0	11.0	28.0
11						4.0
12						2.0
13					3.0	5.0
14			2.0	5.0	16.0	45.0
15			3.0	10.0	25.0	50.0
16			1.0	6.0	8.0	25.0
17					2.0	8.0
18						5.0
19						4.0

*See fig. 6.

**In all sections no disease was in evidence on April 20, 27; May 4, 11, 18 and 25.

ARTIFICIAL INOCULATION TESTS

The virus was obtained for artificial inoculations by two methods. The first method consisted of crushing infected onion leaves in a mortar with sterilized white quartz sand and expressing the juice from the pulp in a hand-press through a thick layer of cotton covered with cheesecloth. The juice was then diluted 1-10 with sterile distilled water and centrifuged for 1 hour at 8,000 R.P.M. The juice, which was used for inoculation purposes was poured off, leaving the greater part of the chlorophyll and coarse leaf tissue in the tubes. In certain tests, however, the centrifuged onion juice from infected onions was passed through a Berkfeld W. filter before inoculations were made. Onion juice from healthy bulbs was similarly treated and used to inject into check plants.² Other inoculations were made in which juice was extracted from the fleshy leaves and scale leaves of infected onion bulbs.

The second method consisted of taking diseased tissue aseptically from yellow dwarf infected bulbs. Three or four of the fleshy leaves were removed, and a plug of the sterile tissue was cut from the remaining part of the bulb. This plug was inserted into incisions in healthy bulbs. Before the healthy bulbs were inoculated, they were soaked for 30 minutes in a solution of mercuric chloride (1-500), with an equal volume of 0.5 percent nicotine sulfate, after which the outer two fleshy leaves were peeled off. Plantings were made in 8-inch pots of sterile soil. The base of each pot was embedded in fresh sand, and a bell jar was sealed to the rim of the pot. Each bell jar had a 2-inch hole in the top, covered with silk bolting cloth. Watering of the plants in these tests was done through a tube in the side of the pots. The tube was kept closed when not in use. Except when otherwise indicated, the tests were conducted under open conditions with an ample number of check plants.

Artificial inoculations were conducted by the following methods: (a) Puncturing leaf tissue with a fine-pointed needle; (b) hypodermic injections into the growing tips of healthy bulbs; (c) cutting leaves from healthy onion plants with a sharp scalpel or topping shears smeared with juice or pulp of yellow dwarf infected onion tissue; (d) rubbing the leaves of healthy onion plants with tissue of an infected onion plant; (e) rubbing the leaves of healthy onion plants with fingers smeared with juice from infected onion plants; (f) inserting plugs from infected onion bulbs into incisions in healthy bulbs; (g) watering healthy onion plants with juice extracted from yellow dwarf infected onion plants.

² The healthy onion bulbs used in all of the inoculation tests were Red Globe and Yellow Bottleneck varieties, grown by S. Kennedy of Clear Lake, Iowa, and R. Workman of Lansing, Ill.

NEEDLE PUNCTURE INOCULATIONS OF ONION LEAVES

Oct. 6, 1929, 20 healthy Red Globe onion plants 2-3 inches in height were inoculated by pricking the inoculum into the base of the succulent leaves. The sterile fine-pointed needle was pushed through a drop of virus-containing onion juice into the leaf tissue. At the same time, five healthy onion plants of the same variety to be used for checks were similarly treated except that juice extracted from healthy onion bulbs was used. The plants were grown in 8-inch pots of steamed soil and placed in a greenhouse containing no other onion plants. Similar inoculation experiments were conducted at nine other times; the results are recorded in table 4.

The 20 onion plants in each of the tests made Oct. 8 and 22, and Nov. 10 and 14 in 1929 and on Feb. 6, 1930, failed to show yellow dwarf. The 20 onion plants, inoculated by this method Oct. 6 and 30, and Nov. 4, in 1929, showed 5 percent infection in each test. Ten percent of the plants in the needle-puncture inoculation tests initiated Oct. 25, 1929, and Feb. 4, 1930, manifested yellow dwarf symptoms. The 10 check plants used in these two tests remained healthy.

Data given in table 4 show that the needle-punctured method of inoculating onion plants with the yellow dwarf virus either had no apparent effect during the first growth period or caused yellow dwarf in only one or two plants.

MODIFIED NEEDLE PUNCTURE METHOD

Oct. 10, 1929, 20 healthy Yellow Globe onion plants, 2-3 inches in height, were inoculated with juice containing yellow dwarf virus by folding a small piece of cotton saturated with this juice around the base of the succulent leaves and pricking the leaf tissue through the cotton with a sterilized, finely pointed needle. About 20 needle punctures were made into each leaf. The saturated cotton wrapper was allowed to remain on the wound of the leaf for two days. At the same time, 10 healthy

TABLE 4. RESULTS OF THE NEEDLE PUNCTURE METHOD OF INOCULATING ONION PLANTS WITH YELLOW DWARF VIRUS.

Date inoculated	Percentage infected		Incubation period
	Virus inoculated (20 healthy bulbs used)	Checks (5 healthy bulbs used)	
Oct. 6, 1929	5		14
Oct. 8, "	0	none	0
Oct. 22, "	0	in-	
Oct. 25, "	10	fected	16
Oct. 30, "	5		16
Nov. 5, "	5		14
Nov. 10, "	0		0
Nov. 14, "	0		
Feb. 4, 1930	10		14
Feb. 6, "	0		0

plants were similarly treated in the leaves with juice extracted from healthy onion bulbs. A total of 12 tests using this method was made on different dates. The same number of bulbs was used in each test, excepting those started Oct. 15, 16 and 20, 1930, in which exactly half the usual number was used. The results obtained when these plants were grown in 8-inch pots of steamed soil are shown in table 5.

TABLE 5. RESULTS OF INOCULATING ONION PLANTS WITH THE YELLOW DWARF VIRUS BY THE MODIFIED NEEDLE PUNCTURE METHOD.

Date inoculated	Number plants inoculated		Percent yellow dwarf		Incubation period
	Virus	Checks	Virus	Checks	
Oct. 10, 1929	20	10	30	none	12
Oct. 12, "	20	10	25	in-	12
Oct. 16, "	20	10	30	fected	12
Oct. 20, "	20	10	35		10
Nov. 10, "	20	10	20		12
Nov. 15, "	25	10	24		10
Nov. 20, "	20	10	40		14
Jan. 15, "	20	10	25		13
Jan. 20, "	20	10	20		12
Oct. 15, "	10	5	30		12
Oct. 16, "	10	5	30		14
Oct. 20, "	10	5	40		10

The data in table 5 show that consistent positive results were obtained in these 12 tests, when healthy onion plants were inoculated in the leaves with yellow dwarf virus by the modified needle-puncture method. The check plants, injected by the same method with healthy onion juice, remained healthy in each of the tests.

In these tests, the period of incubation of the yellow dwarf virus in the onion plants was about 12 days.

HYPODERMIC NEEDLE INOCULATION

The inoculum was injected into the growing points of the healthy onion bulbs with a sterilized hypodermic syringe having a needle 1-inch long. The needle was forced into the bulb from the side, slanting downward into the meristematic tissue, to the center of the bulb plate. At the start of the withdrawal, 1/32 cc. of the inoculum was injected into the growing point and surrounding tissue of the bulb. Two injections were made into each bulb from opposite sides.

This method was tested 16 times in 1929 and 1930, inoculating a total of 340 healthy Red Globe onion bulbs. Half of this number was hypodermically inoculated in the growing point with non-filtered juice, and the other half with juice which had been passed through a Berkfeld W. filter. For the checks in each test, 10 healthy Red Globe onion bulbs were hypodermically injected in the growing points with juice extracted from healthy

onion bulbs. The bulbs were planted in 8-inch pots of steamed soil and placed in a greenhouse. In 6 of the 16 tests the plants were enclosed by sealing bell jars to the tops of the pots. The results are presented in table 6.

TABLE 6. RESULTS OF INOCULATING ONION PLANTS WITH THE YELLOW DWARF VIRUS BY THE HYPODERMIC METHOD.

Date inoculated	Treatment of juice	Condition of tests	Number of bulbs	Percentage yellow dwarf		Incubation percentage
			Inoculated	Inoculated	Checks	
Oct. 4, 1929	Non-filtered	In open	20	85	0	12
Oct. 6, "	"	"	20	90	10	12
Oct. 30, "	"	"	20	85	healthy	12
Nov. 15, "	"	Under bell jars	20	80	bulbs	14
Sept. 29, 1930	"	In open	20	100	used	12
Oct. 15, "	"	"	20	80		14
Oct. 15, "	"	Under bell jars	25	90		12
Dec. 10, "	"	"	25	100		10
Oct. 4, 1929	Filtered	In open	20	70		12
Oct. 6, "	"	"	20	70		14
Oct. 30, "	"	"	20	80		12
Nov. 14, "	"	Under bell jars	20	80		10
Sept. 28, 1930	"	In open	20	70		13
Oct. 15, "	"	"	20	75		12
Oct. 15, "	"	Under bell jars	25	75		12
Dec. 10, "	"	"	25	60		12

Data given in table 6 show that in 16 tests of this method in which 340 onions were inoculated, 60 to 100 percent visible infection resulted. The 10 healthy Red Globe bulbs that were injected hypodermically with juice from healthy onions remained healthy in each test.

Inoculations with the nonfiltered viriferous juice gave somewhat larger percentages of infection in these tests than the inoculations with filtered juice. Growing the inoculated plants under bell jars did not affect the expression of yellow dwarf symptoms. The incubation period of the virus in plants that showed yellow dwarf symptoms ranged from 10 to 14 days.

The hypodermic method of transmitting the virus to onion plants produced more diseased plants than did any other artificial method.

INOCULATIONS WITH JUICE FROM FLESHY AND SCALE LEAVES OF INFECTED BULBS

In 1932, four tests were made in which juice extracted from the two outer fleshy leaves of infected onion bulbs was hypodermically injected into the meristematic tissue of healthy onion bulbs and two tests in which healthy onion bulbs were similarly inoculated with the filtrate of the dry scale leaves of infected bulbs that had been crushed in water. The infected bulbs from which the fleshy and dry scale leaves were taken were planted

as a check on the inoculum in each test. In each test juice from healthy onion bulbs was hypodermically injected into healthy bulbs for checks.

No plants exhibited symptoms of yellow dwarf when fleshy or dry scale leaves of yellow dwarf plants were used as the source of inoculum and the hypodermic method was used. The infected bulbs from which the fleshy and dry scale leaves were taken produced plants that expressed symptoms of the disease in each test. Yellow dwarf virus must have been very dilute in the storage tissue of the infected onion bulbs and inactivated in the dry scale leaves of the bulb and, therefore, caused no infection of the inoculated plants.

As shown by Henderson (8), healthy onion plants inoculated with yellow dwarf virus diluted 1-10,240 or more, and the filtrate from dried infected onion leaves failed to cause infection.

In making hypodermic inoculations of yellow dwarf virus into healthy onion bulbs it has been found important that the virus be injected into the meristematic tissue and not into the surrounding storage tissue if symptoms of yellow dwarf are to be produced. In many instances, however, where bulbs were hypodermically inoculated with the virus, and the plants failed to manifest the symptoms during the current growth period, the yellow dwarf symptoms readily appeared when these bulbs were regrown. In such instance it is probable that the virus was injected in the storage tissue and did not reach the meristematic tissue during the first growth period. However, the virus remained viable, and by the time the bulb had gone through a dormant period and was regrown the virus had found its way into the meristematic tissue. Yellow dwarf symptoms then appeared in the plant. In other instances where the injections of virus were made into the storage tissue, the plants did not show symptoms of the disease regardless of the number of times the bulbs were regrown.

EFFECT OF RATE OF GROWTH ON MANIFESTATION OF YELLOW DWARF SYMPTOMS

Oct. 18, 1930, 1,350 healthy Yellow Globe onion sets were planted in a greenhouse bed. At planting time 80 of the bulbs were hypodermically inoculated in the growing points with non-filtered juice, extracted from yellow dwarf infected onion plants, and 30 of them were similarly injected with juice extracted from healthy onion bulbs for checks. Later, when the plants reached the heights of 1, 2, 3, 4, 5, 6, 7 and 8 inches, respectively, 80 plants were inoculated in the leaves with virus containing juice by the modified needle-puncture method previously described. At the time each lot of 80 onion plants was inoculated, 30 plants were similarly treated with healthy onion juice to serve as checks. The remaining uninoculated plants

were used to ascertain whether any natural transmission of yellow dwarf occurred where the tests were conducted. Thirty-five days after each lot of plants was inoculated, readings were made of the number of visibly infected plants. The plants were allowed to mature and were harvested March 8, 1931. April 10, 1931, all the bulbs from the virus-inoculated plants and check plants injected with healthy juice, were planted in the field near Pleasant Valley. May 15, 1931, final readings were made of the number of plants showing yellow dwarf in this second growth period. These data, together with the percentages of total infected plants which masked the symptoms during the total growth period, are presented in table 7.

TABLE 7. NUMBERS AND PERCENTAGES OF ONION PLANTS SHOWING SYMPTOMS OF YELLOW DWARF AFTER INOCULATION AT VARIOUS STAGES OF GROWTH.

Height when inoculated	Number plants**		No. plants showing symptoms				Percentage infected plants showing masked symptoms in first growth period	
			First growth period		Second growth period			
	Inoculated	Checks	Inoculated	Checks	Inoculated	Checks	Inoculated	Checks
Bulbs*	80	30	64	0	72	0	11.0	0.0
1 in.	80	30	30		50		40.0	
2 in.	80	30	30		44		31.4	
3 in.	80	30	22		40		44.0	
4 in.	80	30	14		30		54.1	
5 in.	80	30	10		24		58.0	
6 in.	80	30	0		18		100.0	
7 in.	80	30	0		22		100.0	
8 in.	80	30	0		16		100.0	

*Bulbs were hypodermically inoculated in the growing tips with yellow dwarf virus.

**The 270 uninoculated plants remained healthy.

Experiments reported in table 7 on inoculation of onions with yellow dwarf virus at different stages of growth show that (a) the earlier the inoculation, the higher the percentage of infection obtained, (b) although approximately the same percentage of plants inoculated at all stages masked the symptoms during the first growth period, the later the inoculations the lower the percentage of visible infection until the plants were higher than 5 inches, when no visible infection was present in the first growth period, (c) higher percentages of visible infection occurred in the second growth period than in the first because some of the infected plants masked the symptoms in the first growth period, (d) inoculations made when plants approached maturity failed to manifest disease symptoms until the bulbs of such plants were regrown.

Results of these artificial inoculation tests correlate with field conditions, in that plants infected late in maturity by the natural transmission of the yellow dwarf virus either fail to mani-

fest symptoms of the disease, or show symptoms in only the last few leaves.

As was brought out in the previous section on masking of symptoms, the manifestation of yellow dwarf depends on the vigorous growth of the plant after it becomes inoculated.

INOCULATION WITH JUICE FROM APPARENTLY HEALTHY (MASKED YELLOW DWARF) ONION PLANTS

June 28, 1928, 20 apparently healthy Red Globe onion plants were collected from fields on the C. Kramback farm, where 95 percent of the plants expressed yellow dwarf symptoms. Juice was extracted from the leaves of each plant, and lots of 20 healthy Yellow Globe onion sets were hypodermically inoculated in the growing tips with each sample of juice, respectively. For checks, sterile water was injected into the growing tips of a similar number of healthy Yellow Globe onion sets. These bulbs were planted on a farm in a yellow dwarf disease-free area, 4 miles from the nearest onion field. Final readings were taken on these tests July 30, 1928.

Oct. 10, 1929, 50 healthy Yellow Globe onion plants were hypodermically inoculated in the growing tips with yellow dwarf virus. Twenty-five days later 60 percent of the plants manifested the symptoms. Juice was extracted from each of 15 apparently healthy plants, and lots of 10 healthy Yellow Globe onion bulbs were hypodermically inoculated with each sample of juice, respectively. To serve as checks, lots of 10 healthy Yellow Globe bulbs were similarly treated with sterile water. These bulbs were planted in 8-inch pots and held under greenhouse conditions. Final readings were made Nov. 5, 1929. Data from both the 1928 and 1929 experiments are given in table 8.

Data in table 8 show that juice extracted from infected onion plants which had masked yellow dwarf symptoms was infectious.

Ten to 50 percent of the onion plants inoculated with juice from the apparently healthy plants from the Kramback field, expressed yellow dwarf symptoms.

Thirty to 50 percent of the onion plants inoculated with juice from the apparently healthy plants of the greenhouse inoculation test plants 1, 2, 3, 4, 6, 7, 10, 13, 14, and 15 showed yellow dwarf symptoms, while those inoculated with juice from the apparently healthy plants 5, 8, 9, 11 and 12 showed no signs of the disease.

Juice extracted from infected onion plants, when used to artificially inoculate healthy plants, caused infection. Under field conditions, therefore, any yellow dwarf infected plant, whether the symptoms are visible, provides a source of inoculum by which healthy plants may be infected.

TABLE 8. PERCENTAGE OF ONION PLANTS THAT MANIFESTED YELLOW DWARF SYMPTOMS WHEN HYPODERMICALLY INOCULATED WITH JUICE EXTRACTED FROM APPARENTLY HEALTHY ONION PLANTS.

Source of inoculum	Number bulbs		Percentage of infected plants	
	Inoculated	Checks	Inoculated	Checks
Kramback plants 1	20	20	35	No infection
" " 2	20	20	30	
" " 3	20	20	35	
" " 4	20	20	45	
" " 5	20	20	35	
" " 6	20	20	30	
" " 7	20	20	45	
" " 8	20	20	50	
" " 9	20	20	30	
" " 10	20	20	40	
" " 11	20	20	20	
" " 12	20	20	35	
" " 13	20	20	45	
" " 14	20	20	10	
" " 15	20	20	40	
" " 16	20	20	30	
" " 17	20	20	40	
" " 18	20	20	40	
" " 19	20	20	30	
" " 20	20	20	35	
Greenhouse test plants 1	10	10	30	
" " " 2	10	10	30	
" " " 3	10	10	50	
" " " 4	10	10	30	
" " " 5	10	10	0	
" " " 6	10	10	40	
" " " 7	10	10	50	
" " " 8	10	10	0	
" " " 9	10	10	0	
" " " 10	10	10	30	
" " " 11	10	10	0	
" " " 12	10	10	0	
" " " 13	10	10	40	
" " " 14	10	10	40	
" " " 15	10	10	50	

POSSIBLE NATURAL DISSEMINATION OF THE VIRUS

The manner in which the yellow dwarf virus is disseminated in the field was not determined. The known infectiveness of certain viruses suggested the desirability of studying the possible role of cultural practices and of insects as vectors. Onion growing employs considerable hand labor, because of large numbers of plants growing in close proximity. The plants are subjected to possible injury in weeding, cultivating and harvesting.

Like other crops, onions are visited and infested with certain insects as thrips, onion maggots, the tarnished plant bug and others. The known role of insects as vectors in other virus diseases suggested their possible relation to the spread of the yellow dwarf virus.

In order to simulate cultural practices, a knife was smeared with juice from yellow dwarf infected plants and used for topping. Ten one-rod rows of healthy onion plants were topped,

when about half grown. The same procedure was followed at harvest time.

In another set of trials the virus infected juice was applied to the hands of laborers who rubbed the leaves of 630 healthy plants. Bulbs of healthy young plants were injured in still other trials. Plugs were taken from infected bulbs and inserted into 250 healthy ones. Later these plants were harvested and regrown in the greenhouse for evidence of yellow dwarf virus. In none of the trials, however, did transmission take place.

Drake, Harris and Tate (3) and Drake, Tate and Harris (4) report that the bean aphid (*Aphis maidis* Fitch), the apple grain aphid (*Rhopalosiphum prunifoliae* Fitch), the green peach aphid (*Myzus persicae* Sulzer), the melon aphid (*Aphis gossypii* Glover) and the potato aphid (*Macrosiphum gei* Koch) are vectors of the yellow dwarf virus. They also have reported that the six-spotted leaf-hopper (*Cicadula sexnotata* Fall) and a mealy bug (*Phenacoccus* sp.) are capable of transmitting the virus.

The author has made a number of controlled experiments on transmission of yellow dwarf virus with the bean aphid (*Aphis maidis* Fitch), the apple grain aphid (*Rhopalosiphum prunifoliae* Fitch), and the melon aphid (*Aphis gossypii* Glover) with successful results. Since this phase of the problem is being investigated in the Entomology Section no further consideration here will be given this aspect of the yellow dwarf problem.

Evidence accumulated on transmission shows that cultural practices probably do not spread the yellow dwarf virus and that certain insects may serve as vectors. -

PROPERTIES OF YELLOW DWARF VIRUS

After the artificial inoculation methods had been perfected so that consistently high percentages of infection could be obtained, experiments were conducted to determine the properties of the yellow dwarf virus. These tests consisted of aging in vitro, drying leaves, thermal death point, and dilution.

AGING IN VITRO

A supply of juice was extracted from leaves of yellow dwarf-infected onions and diluted with 1 cc. of sterile distilled water to each gram of leaves used in the extraction. The liquid was stored in a stoppered sterile glass container at 29° C. It was shown by Henderson (8) that onion virus stored under the above conditions remained infective up to 112 hours. Duplicate lots of 50 healthy bulbs were inoculated by injecting 1/16 cc. of the diluted virus into each bulb. Fifty additional healthy bulbs were injected with sterile distilled water as checks. The tests were started May 14, 1931, and continued at different intervals for 650 hours. The bulbs were planted in a disease-free

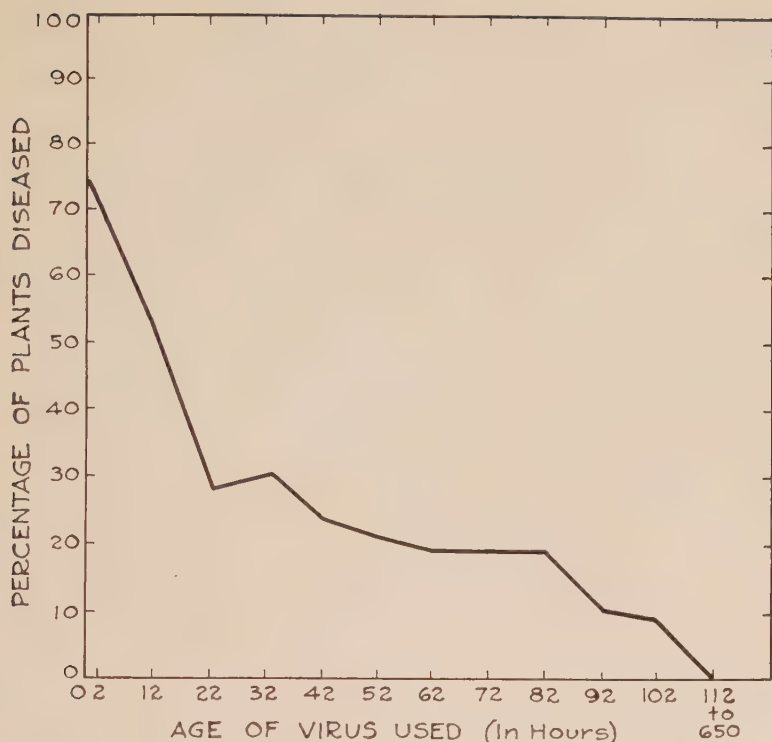


Fig. 7. Effect of age of yellow dwarf virus in vitro on infectivity.

area. In fig. 7 are shown the percentages of infected plants occurring in the virus-inoculated lots made at the different time intervals.

The inoculations made with freshly extracted viriferous juice visibly infected 74 percent of the plants. There was a sharp reduction in the infectivity of the yellow dwarf virus stored for 12 and 22 hours as indicated by 53.0 and 28.0 percent of the inoculated plants that manifested symptoms in the respective tests. There was a gradual loss of infectivity of the stored yellow dwarf virus for the time intervals from 22 to 112 hours, after which inoculations produced no yellow dwarf symptoms.

INFECTIVITY OF THE VIRUS FROM DRIED INFECTED LEAVES

Leaves were collected from yellow dwarf infected onion plants and stored at 29° C. Starting May 14, 1931, at different intervals juice was extracted from the stored leaves and hypodermically injected into 50 healthy onion bulbs. The different trials included time intervals to 180 hours. The bulbs were planted in a disease-free area and read after 22 days. It was

shown by Henderson (8) that yellow dwarf virus in drying onion leaves stored at 29° C. remained infective for 100 hours. In fig. 8 is shown the effect of drying on the infectivity of the yellow dwarf virus.

The onions inoculated with the yellow dwarf virus from freshly cut leaves showed 86.0 percent visible infection. Inoculations with juice from leaves dried 10 hours resulted in 84 percent visible infection.

As shown in fig. 8, the percentage of visible infection decreased with the time the leaves were dried. After 100 hours no infection was obtained. The results indicated clearly that when the leaves were air dry the virus was no longer capable of inducing infection. Results of these viability tests of the virus in the severed leaves are of considerable importance to the grower because onion tops are allowed to remain in the field.

EFFECT OF HEAT ON THE INFECTIVITY OF THE YELLOW DWARF VIRUS

Juice was extracted from diseased Yellow Bottleneck onion

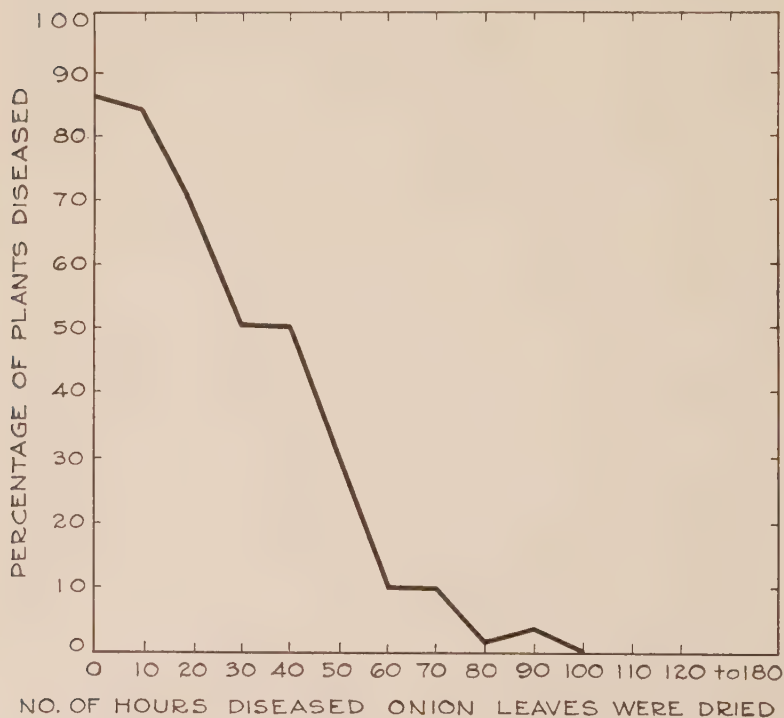


Fig. 8. Infectivity of the yellow dwarf virus from infected leaves in the process of drying.

plants by the method previously described and diluted with 1 cc. of sterile distilled water for each gram of leaves. The virus-containing juice was filtered and placed in thin sterile glass tubes $\frac{1}{8}$ inch in diameter and $1\frac{1}{2}$ inches long. The glass tubes were stoppered with sterilized corks, and placed in a wire basket covered with a wire screen so as to hold them upright. Immediately before exposure in the constant temperature water bath to temperatures of 60, 70, 75, 80, 85, 90 and 95° C, for 10 minutes, the basket and tubes of material were immersed for 1 minute in a water bath of similar temperatures so that the materials would be heated to the correct temperature to begin the tests. Tests were made in duplicate and terminated by immersing the basket for 5 minutes in cold water. In each test 50 healthy Yellow Globe onion bulbs were hypodermically inoculated with $\frac{1}{16}$ cc. of the previously heated viriferous onion juice. At the same time 50 healthy Yellow Globe onion bulbs for each test were similarly injected with previously heated healthy onion juice as checks. As a check on the infectivity of the virus 50 healthy Yellow Globe onion bulbs in each test were hypodermically inoculated with non-heated virus-containing juice from the same supply of onion juice as used in the temperature test. Onion bulbs of these tests were inoculated immediately after each temperature treatment was completed and planted in a disease-free area. Each test was read 25 days after the date of planting. In fig. 9 the percentages of visible infection found in the bulbs inoculated with heated viriferous onion juice and non-heated viriferous juice are shown.

Figure 9 shows that heating the yellow dwarf virus for 10 minutes at 60 and 70° C. had little or no effect upon its infectivity. Heating for 10 minutes at 75° C., however, reduced the infectivity of yellow dwarf virus quite sharply, and heating at 80° C. for 10 minutes destroyed its infectivity. Percentages of infection found in the check plants that were inoculated with the non-heated yellow dwarf virus were uniformly high. The critical temperature for the yellow dwarf virus, therefore, seems to lie between 75 and 80° C. when exposed 10 minutes. [Henderson (8)].

EFFECT OF LOW TEMPERATURE ON THE INFECTIVITY OF THE YELLOW DWARF VIRUS

Juice was extracted from leaves of onion plants infected with yellow dwarf, diluted 1-10 with sterile distilled water, centrifuged for 1 hour, at 8,000 r.p.m., and placed in small quantities in sterilized flat-bottomed pans. The containers of diseased onion juice were then placed out-of-doors, for different lengths of time, on a day when the temperature was below zero (centigrade). This resulted in exposures of 15 minutes at -5° C., 30 minutes at -10° C., 2 hours at -14° C., and 6 hours at -14° C.,

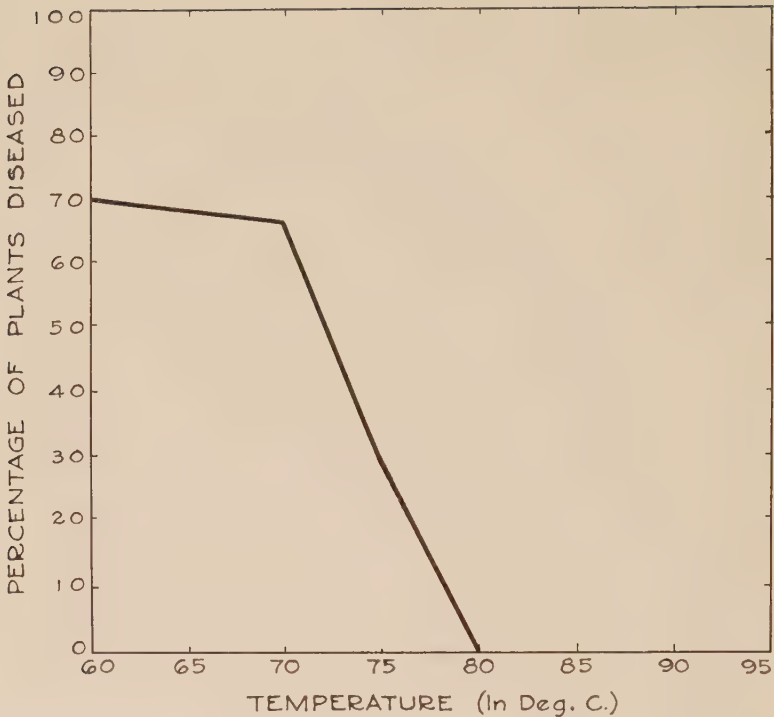


Fig. 9. Effect of heat on infectivity of the yellow dwarf virus.

respectively. Low temperature exposures were terminated by bringing the materials into a room, the temperature of which was about 25° C. Duplicate tests of the infectivity were made by hypodermically inoculating healthy onion bulbs with the virus treated in the ways mentioned. As a check on the test of cold exposures 20 healthy onion bulbs were hypodermically injected with healthy onion juice that had been exposed to the same conditions as the virus-containing juice and 10 healthy onion bulbs were hypodermically inoculated with the untreated virus from the same original supply as used in the cold exposure tests. The bulbs were planted in 8-inch pots of soil, and placed in a greenhouse separate from other onion plants. (Data from these experiments are shown in table 9.)

Data in table 9 show that the exposures to freezing temperatures in these experiments, which ranged up to 6 hours at -14° C. had no effect on the infectivity of the yellow dwarf virus. [Henderson (8)].

YELLOW DWARF VIRUS DILUTION TESTS

Allard (1) showed that the virus-containing tobacco juice diluted 1-1,000 was quite as effective in producing infection as

the undiluted juice, but that the 1-10,000 dilution reduced the percentage of infection. It seemed desirable to make similar tests to determine the effect of dilutions on infectivity of the yellow dwarf virus.

Juice was extracted from yellow dwarf infected Red Globe onion plants, strained through cotton, centrifuged for 30 minutes and passed through a Berkfeld W. filter. Inoculations were made by injecting 1/16 cc. of the filtered diseased juice into the growing points of 20 healthy Red Globe onion bulbs, in duplicate tests. Similar inoculation tests were made with each of the dilutions which ranged up to 1 part undiluted diseased juice in 163,640 parts sterile distilled water. In each of the dilution tests, 10 healthy Red Globe onion bulbs were injected hypodermically with sterile distilled water for checks. The bulbs were planted in 8-inch pots of soil and placed in a greenhouse separate from other onion plants. Readings of the percentage yellow dwarf in each test were made 24 days after planting, and the data are shown graphically in fig. 10.

The data presented in fig. 10 show that dilutions up to 1 in 20 all infected the healthy plants about alike, in percentages ranging from 83 to 96. For greater dilutions, the percentage of infected plants decreased uniformly with doubling of the amount of water. At a dilution of 1 in 10,240 and beyond, no healthy plants were infected by the diluent. The check plants inoculated with sterile distilled water remained healthy in each of the tests.

TABLE 9. NUMBER AND PERCENTAGE OF DISEASED PLANTS IN LOTS INOCULATED WITH HEALTHY AND VIRUS-CONTAINING JUICE EXPOSED TO LOW TEMPERATURES.

Exposure time	Temp pera- ture °C.	Yellow dwarf plants			
		Healthy-juice check		Virus-containing juice	
		Number	Percent	Number	Percent
15 min.	- 5	0	0	24	80
15 min.	- 5	0	0	25	83
No exposure	—*	—	—	14	70
	—	—	—	12	60
30 min.	-10	0	0	23	76
30 min.	-10	0	0	27	90
No exposure	—*	—	—	18	90
	—	—	—	18	90
2 hours	-14	0	0	24	80
2 hours	-14	0	0	25	83
No exposure	—*	—	—	20	100
	—	—	—	16	80
6 hours	-14	0	0	27	90
6 hours	-14	0	0	24	80
No exposure	—*	—	—	18	90
	—	—	—	20	100

*Room temperature.

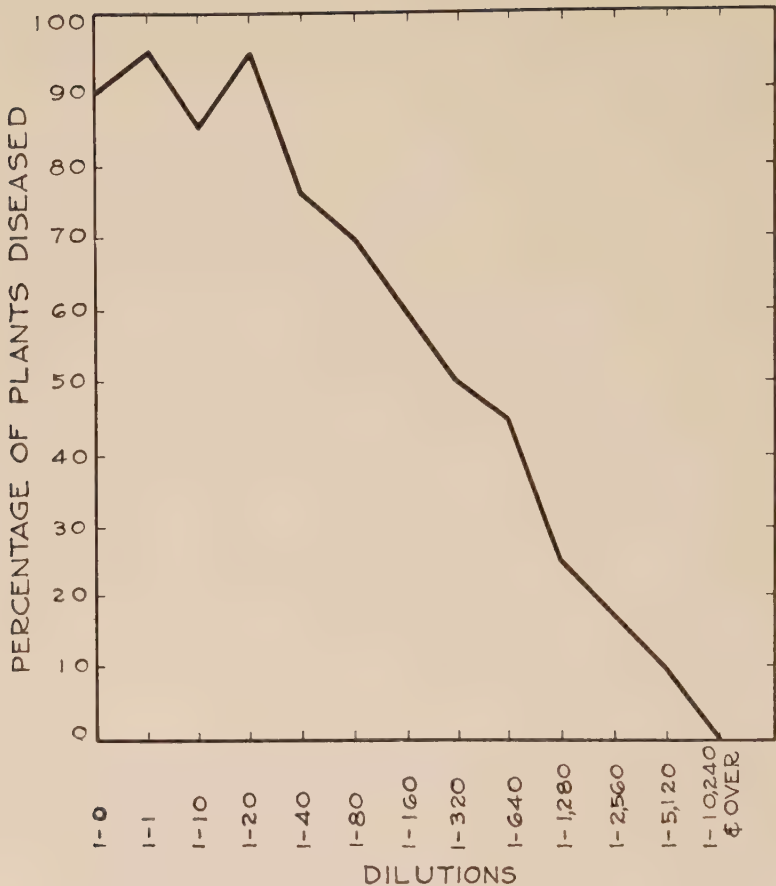


Fig. 10. Effect of dilution on the infectivity of yellow dwarf virus.

Concentration of the yellow dwarf virus in the onion plant was not as great as in the case of the tobacco mosaic and cucumber mosaic virus, as indicated when dilutions of 1-5,120 infected only 10 to 30 percent of the inoculated plants.

OVERWINTERING OF THE YELLOW DWARF VIRUS

To determine the manner in which the yellow dwarf virus overwintered, tests were conducted in the field and greenhouse with soil from the diseased area, seed from diseased plants, bulbs from diseased plants, and diseased plants remaining outside during the winter.

IN SOIL

Soil tests were conducted under greenhouse conditions at

Ames, Iowa, to determine whether the virus was soil-borne, as was found in the case of wheat rosette by McKinney (11).

In the fall of 1928 samples of soil were collected from the Kramback farm at Pleasant Valley, Iowa, where 95 percent of the previous crop had been visibly infected. One hundred and twelve Red Globe onion seedlings and two hundred healthy Yellow Globe onion sets, two varieties known to be highly susceptible to yellow dwarf, were planted in 8-inch pots containing soil from the Kramback farm. For checks 32 Red Globe onion seedlings and 50 healthy Yellow Globe onion sets were planted in 8-inch pots of the soil from the Kramback farm that had been steamed at 28 pounds pressure for 3 hours per day on two consecutive days.

Similar tests were conducted where 72 Red Globe onion seedlings and 110 healthy Yellow Globe onion sets were planted in 6-inch pots of compost soil, where infected onions had previously grown under greenhouse conditions. Twenty-eight Red Globe onion seedlings and forty healthy Yellow Globe onion sets were planted as checks in soil obtained from the same source, which had been steamed as above. None of the plants from sets or seed became infected, which indicates that the virus is not soil borne.

IN SEED

During the spring of 1928 (before the nature of the onion trouble was known) a large quantity of Red Globe onion seed from a diseased field at Pleasant Valley, Iowa, was sold to onion growers of the St. Ansgar, Sumner, Washington, and Clear Lake, Iowa, and the Hollandale, Minn., disease-free districts. A total of 75 acres, including 2 acres at the horticultural farm at Ames, Iowa, were planted with this seed. When the plants of these crops were 12-14 inches in height, and for the remaining part of the growing season, careful inspections were made for the presence of yellow dwarf. Data obtained are given in table 10.

Red Globe seed produced by infected onion plants and planted on 73 acres in the St. Ansgar, Sumner, Washington, and Clear Lake, Iowa, and Hollandale, Minn., districts produced

TABLE 10. DATA ON THE EFFECT OF USING SEED FROM YELLOW DWARF DISEASED PLANTS.

Seed from diseased plants sown at:	Number acres	Percentage of plants visibly infected
St. Ansgar, Iowa	51	0.0
Sumner, Iowa	2	0.0
Washington, Iowa	8	0.0
Clear Lake, Iowa	2	0.0
Ames, Iowa	2	15.0
Hollandale, Minn.	10	0.0

crops of healthy onions. Fifteen percent of the plants from the same Red Globe onion seed lot planted on a 2-acre plot at Ames, showed yellow dwarf. Because the Ames plot comprised only 2 of the 75 acres planted with seed from the same lot indicates that this was an exceptional occurrence and that the infection probably was not carried in the seed.

July 25, 1928, samples of Yellow Bottleneck and Red Globe onion seed were collected from visibly infected plants growing on two farms near Pleasant Valley, Iowa. Feb. 10, 1929, 60 seeds of the Yellow Bottleneck variety and 64 seeds of the Red Globe variety were planted in 8-inch pots of steamed compost soil. Twenty-five Yellow Bottleneck and thirty Red Globe seeds produced by healthy onion plants were planted in 8-inch pots of steamed compost soil and held as checks. The plants were grown under greenhouse conditions to the height of 4 inches, when they were placed outside the greenhouse. June 10, 1929, when final readings were made all the plants of these tests were healthy. The data presented in table 10 and the results of growing the seed from infected plants in the greenhouse indicate that the yellow dwarf virus is not seed-borne.

IN BULBS

In the fall of 1928 diseased plants from crops of Yellow Bottleneck sets and mature onions intended for mother bulbs were collected from four farms at Pleasant Valley, Iowa. March 28, 1929, these bulbs were planted in 6-inch flats of sterile soil and held in a greenhouse separate from other onion plants. Healthy Yellow Globe bulbs obtained outside the yellow dwarf area were planted under similar conditions as checks. Final readings of these tests were made June 10, 1929.

It was shown that without exception the 200 mother bulbs and the 200 sets produced by visibly infected plants on four farms at Pleasant Valley, Iowa, showed yellow dwarf symptoms when they were regrown in flats of sterile soil in the greenhouse. In the same experiment Yellow Globe bulbs produced outside of the diseased area by healthy plants remained healthy when regrown. These results show that the yellow dwarf virus was carried over winter in the onion bulbs.

IN VOLUNTEER ONION PLANTS

Late in the fall of 1928, a large number of yellow dwarf volunteer onion plants was found growing in the onion fields, as the result of the practice of scattering defective bulbs over the fields and plowing them under. In the spring of 1929, although the tops of these onions had frozen to the ground, the bulbs and base of the leaves which had been buried deeply in plowing, remained alive and showed yellow dwarf when new growth

started in the spring. Juice extracted from leaves of overwintered infected onion plants was used to inoculate healthy Yellow Globe onion bulbs. Healthy Yellow Globe bulbs were likewise injected with sterile water as checks in each case. The tests in 1929 and in 1930 were planted near Pleasant Valley, but outside the diseased area at least 4 miles from any onion field.

Additional material was obtained by growing a plot of yellow dwarf diseased onions at Ames in 1929. March 16, 1930, after finding that about 2 inches of the tops of these plants were alive, 20 healthy Red Globe onion bulbs were hypodermically inoculated in the growing tips with juice extracted from the leaves of these plants. Another set of 30 healthy Red Globe onion bulbs was likewise inoculated March 20, 1930, and 10 healthy Red Globe onion bulbs were injected with sterile water as checks. All of these bulbs were planted in 8-inch pots of steamed soil and held in a greenhouse where other onions were absent. The results are recorded in table 11.

TABLE 11. PERCENTAGE OF PLANTS SHOWING YELLOW DWARF WHEN HEALTHY BULBS WERE HYPODERMICALLY INOCULATED WITH THE YELLOW DWARF VIRUS WHICH HAD OVERWINTERED IN VOLUNTEER ONION PLANTS.

Source of infected volunteer plants	Date inoculated	Number bulbs		Percentage visible infection	
		Inoculated	Check	Inoculated	Check
Pleasant Valley	May 15, 1929	50	25	68	0.0
" "	May 15, 1929	50	25	76	0.0
" "	May 10, 1930	25	10	60	0.0
" "	May 10, 1930	25	10	80	0.0
Ames	March 16, 1930	20	10	60	0.0
"	March 20, 1930	30	10	53	0.0

Data given in table 11 show that the yellow dwarf virus lived overwinter in the bulbs and leaves of volunteer onion plants. Fifty-three to eighty percent of the onion plants inoculated with yellow dwarf virus that had overwintered in volunteer onions manifested yellow dwarf symptoms. Check plants in these tests remained healthy.

Results of these experiments show that the yellow dwarf virus was neither soil borne nor probably seed borne—at most only in exceptional cases—but was harbored in the vegetative organs of onion plants such as sets, mother bulbs and volunteers in the field.

These overwintering studies warranted the initiation of a new cultural practice—that of indexing sets and mother bulbs and destroying all volunteers. This topic will be described and discussed again in the section on control (page 248).

INTER-TRANSMISSIBILITY OF THE YELLOW DWARF VIRUS

To combat effectively the spread of the yellow dwarf virus,

its host range needed to be known. Experiments, therefore, were conducted in which plants of different genera were inoculated with the yellow dwarf virus from onions. Also onion plants were inoculated with different plant viruses. These tests were made in the greenhouse and in the field by artificial inoculations and with insects. In each test were held check plants which were treated in the same way, using healthy juice.

Shallots (*Allium ascolanicum* L.), Chinese sacred lily (*Narcissus tazetta* L.), and the jonquil (*Narcissus jonquilla* L.) were the only plants tested that showed yellow dwarf symptoms following inoculation with the virus. The other plants inoculated were: Milkweed (*Asclepias syriaca* L.), pigweed (*Amaranthus retroflexus* L.), sugar beet (*Beta vulgaris* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita moschata* Duchesne), lamb's quarter (*Chenopodium alba* L.), corn (*Zea mays indentata* L. Bailey), Mexican dropseed (*Muhlenbergia mexicana* L. Trin.), yellow foxtail (*Setaria glauca* L. Beauv.), gladiolus (*Gladiolus* sp. L.), alfalfa (*Medicago sativa* L.), bean (*Phaseolus vulgaris* L.), red clover (*Trifolium pratense* L.), sweet clover (*Melilotus alba* (Desr.)), Persian iris (*Iris persica* L.), chives (*Allium schoenoprasum* L.), garlic (*Allium sativum* L.), leek (*Allium porrum* L.), nebuka, wild onion (*Allium canadense* L.), Easter lily (*Lilium longiflorum* L.), tiger lily (*Lilium tigrinum* Ker.), golden banded lily (*Lilium auratum* Lindl.), *Lilium speciosum* Thunb., madonna lily (*Lilium candidum* L.), regal lily (*Lilium regale* Wels.), common hyacinth (*Hyacinthus orientalis* L.), *Narcissus poeticus* L.), plantain (*Plantago major* L.), canna (*Canna generalis* Bailey), tulip (*Tulipa gesneriana* L.). These showed no yellow dwarf symptoms.

INOCULATION OF ONION PLANTS WITH OTHER PLANT VIRUSES

In these experiments onion plants were hypodermically inoculated with juice extracted from different kinds of virus-diseased plants. The check plants which were injected with healthy plant juice corresponding to the plants from which the virus was extracted were:

Sweet clover (*Melilotus alba* Desr.), alfalfa (*Medicago sativa* L.), raspberry (*Rubus occidentalis* L.), tobacco (*Nicotiana tabacum* L.), cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.), milkweed (*Asclepias syriaca* L.), bean (*Phaseolus vulgaris* L.), Easter lily (*Lilium longiflorum* L.), sugar cane (*Saccharum officinarum* L.), tulip (*Tulipa gesneriana* L.), pepper (*Capsicum frutescens* L.), and canna (*Canna generalis* Bailey). None of these plants produced symptoms of yellow dwarf in the inoculated healthy onion plants.

ONION PLANTS ARTIFICIALLY INOCULATED WITH JUICE FROM WEEDS IN ONION FIELDS

Experiments were conducted to determine the possibility of wild host plants harboring yellow dwarf virus. Healthy onion bulbs were inoculated hypodermically in the growing tips with juice extracted from plants (not onions) collected in and near diseased onion fields at Pleasant Valley, Iowa, regardless of whether the plants showed signs of a virus disease. At the same time, healthy bulbs were injected with sterile distilled water for checks. These bulbs were planted in the field in an isolated disease-free area.

None of the weeds tested carried yellow dwarf virus. They were: Sweet clover (*Melilotus alba* Desr.), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), wild lettuce (*Lactuca canadensis* L.), lamb's quarter (*Chenopodium album* L.), quack grass (*Agropyron repens* L. Beauv.), velvet weed (*Holcus lanatus* L.), yellow foxtail (*Setaria glauca* L. Beauv.), pigweed (*Amaranthus retroflexus* L.), plantain (*Plantago major* L.), old witch grass (*Panicum capillare* L.), Mexican dropseed (*Muhlenbergia mexicana* L.) barnyard grass (*Echinochloa crus-galli* L.), wild onion (*Allium canadense* L.), and purslane (*Portulaca oleracea* L.).

CONTROL MEASURES

The prevalence and destructiveness of yellow dwarf disease, when this problem was undertaken, made it necessary to give serious consideration to control measures at the outset. It was decided to study the value of spraying as a means of decreasing the spread of the virus and to investigate the possibility of indexing in an effort to eliminate planting stocks of infected bulbs. Because of the marked prevalence (40 percent infection in the Valley) it appeared that healthy sets could not be grown in or near the infected fields. This led to the initiation of the practice of growing or having grown outside the Valley in yellow dwarf free areas all sets to be used for commercial crops within the diseased area. Subsequently, sanitation and varietal resistance also received consideration.

THE EFFECT OF SPRAYING ON THE SPREAD OF YELLOW DWARF INFECTION

The prevalence of yellow dwarf throughout the onion-growing district suggested that the virus was being spread by some unknown insect vector. This led to trials with various spray mixtures on onion seedlings for sets from 1928 to 1931, inclusive.

1928 EXPERIMENTS

In 1928 six plots were laid out on the farm of R. M. Rice, located near the center of the infected area. Each plot consisted of six 150-foot rows of Yellow Bottleneck onions. Spray-

ing was started June 16, using a wheelbarrow sprayer which delivered the spray under 35 pounds pressure. In plot 1, a 4-4-50 Bordeaux mixture, to which was added 1 gallon of furfural and $\frac{1}{4}$ pint of nicotine sulfate to each 50 gallons, was applied for 18 consecutive days.

The same kind of spray was applied at 3-day intervals for 27 days in plot 2. In plot 3 the same kind of spray was applied at 6-day intervals for 24 days. Plot 4 was sprayed daily for 18 days with a solution of soap and nicotine sulfate, made by adding 5 bars of white laundry soap and $\frac{1}{4}$ pint of nicotine sulfate to 50 gallons of water. In plot 5, the plants were sprayed with the same kind of spray used in plot 4, at 3-day intervals for 27 days. The plants in plot 6 were sprayed with the same spray used in plots 4 and 5, at 6-day intervals for 24 days.

Final readings on the percentage of infected plants in each plot were based on 5,000 seedling plants.

July 25 sample lots of 500 sets were collected at random from each plot, and 200 from each check, respectively. Nov. 6, 200 sets from each sample were planted in greenhouse beds for indexing. Effectiveness of the different sprays under these conditions is shown in table 12.

TABLE 12. PERCENTAGE OF VISIBLY INFECTED PLANTS IN ONION SEEDLINGS FOR SETS SPRAYED AT DIFFERENT INTERVALS IN 1928.

Spray	Plot number	Appli- cation intervals days	No. appli- cations	Percentage visible infection			
				In field		When indexed	
				Sprayed	Unspray- ed checks	Sprayed	Unspray- ed checks
Bordeaux mixture, nico- tine sulfate and fur- fural	1	daily	18	0.0	0.0	1.3	18.0
	2	3	9			2.6	18.0
	3	6	4			2.6	18.0
Water, soap and nicotine sulfate	4	daily	18			1.3	18.0
	5	3	9			1.3	18.0
	6	6	4			2.6	18.0

Onion seedlings sprayed with Bordeaux mixture (4-4-50), furfural and nicotine sulfate were severely burned. Those sprayed with the soap and nicotine sulfate solution at the more frequent intervals, were slightly burned at the leaf tips.

Table 12 shows that spraying onion seedlings gave promise of control of the spread of yellow dwarf virus in the set crop, as indicated by the results obtained in the indexing tests. The different procedures with these sprays reduced the infection from 18 percent to percentages ranging from 1.3 to 2.6.

TABLE 13. DATA ON SPRAYING AND PERCENTAGE INFECTION OF ONIONS GROWING FOR SETS.

Spray used	Number		Applica- tion inter- vals days	No. appli- ca- tions	Percentage of visible infection			
					Sprayed		Unsprayed checks	
	Plot	Series			Field	Index	Field	Index
Bordeaux mixture and nico- tine sulfate	1	1	3	18	0.0	0.2	0.0	7.0
"	2	1	6	9	0.0	3.5	0.0	7.0
"	3	1	9	6	0.0	4.0	0.0	7.0
Water, rosin fish-oil soap and nicotine sulfate	4	1	3	18	0.0	1.5	0.0	7.0
"	5	1	6	9	0.0	2.0	0.0	7.0
"	6	1	9	6	0.0	4.0	0.0	7.0
Bordeaux mixture and nico- tine sulfate	1	2	3	18	0.0	3.0	0.0	7.0
"	2	2	6	9	0.0	3.0	0.0	7.0
"	3	2	9	6	0.0	2.0	0.0	7.0
Water, rosin fish-oil soap and nicotine sulfate	4	2	3	18	0.0	1.0	0.0	7.0
"	5	2	6	9	0.0	3.0	0.0	7.0
"	6	2	9	6	0.0	2.0	0.0	7.0

1929 EXPERIMENTS

In 1929 a duplicate series of spray plots was established, one on each of two farms. In the second series the rows were only half as long as in the first. Spraying was started June 1, using 40 pounds pressure. Plot 1 in each series was sprayed at 3-day intervals with 4-4-50 Bordeaux mixture, to which was added $\frac{1}{4}$ pint of nicotine sulfate to each 50 gallons; plot 2 at 6-day intervals; and plot 3 at 9-day intervals. The same spray schedule was carried out in sections 4, 5 and 6, in both series of plots but the spray consisted of 2 pounds of rosin fish oil soap and $\frac{1}{4}$ pint of nicotine sulfate for each 50 gallons of water. The spray schedule was followed for 54 days.

Final readings were made June 30, and the sets were harvested July 25. Sept. 30 sample lots of 200 sets taken from each plot were planted in the greenhouse for indexing.

Severe burning occurred when Bordeaux mixture was used, but only slight tip burning occurred where the rosin fish oil soap and nicotine sulfate were applied. No visible infection was found in the field on sprayed or unsprayed seedlings, but after plants were harvested and the sets indexed, it was found that the yellow dwarf symptoms had been masked. Infection occurred in all the sprayed and unsprayed plots, as determined by visible symptoms brought out by indexing. There was no significant difference in the percentages of infection where the different sprays were used, but both spray mixtures reduced the percentage of infection (table 13).

1930 AND 1931 EXPERIMENTS

In 1930 four 150-foot rows of onions grown from sets were sprayed at 7-day intervals for 29 days with Bordeaux mixture to which was added 1 gallon of white oil emulsion and $\frac{1}{4}$ pint of nicotine sulfate. Two adjacent rows were left unsprayed as checks.

In 1931 a duplicate series of spray plots was located one on each of two farms. Bordeaux mixture (4-4-50) to which was added 1 gallon of white oil emulsion and $\frac{1}{4}$ pint of nicotine sulfate for each 50 gallons of mixture was used on the first plots of each series. It was applied at 6-day and 9-day intervals with two replications of each. A spray consisting of 1 gallon of white oil emulsion, 400 cc. of liquid C.P.O. soap and $\frac{1}{4}$ pint of nicotine sulfate to each 50 gallons of water was applied in a similar manner to plot 2 of each series. The schedules continued for 60 or 63 days as best fitted the intervals.

No yellow dwarf symptoms were observed in the spray plots in either year. However, when 300 sets from each plot in 1930 were indexed, 5 percent in the sprayed plots were found to be infected and 6 percent in the check plots. Indexing 200 sets from each plot in the 1931 tests showed zero to 2.0 percent infection in both the sprayed and unsprayed plots. The low percentage of infection in both sets of plots indicated a very limited dissemination of the yellow dwarf virus in 1931, and that these spray schedules failed to control it.

From the results obtained in the spray experiments for the 4 years it is evident that some control of field spread of the yellow dwarf virus may be obtained by the use of the sprays. In all trials where Bordeaux mixture was used, the plants were severely burned, and the other spray mixtures caused slight tip burning. Because of application cost and the limited control of the spread of the virus, the value of the spray is doubtful.

VARIETAL RESPONSE TO YELLOW DWARF INFECTION

In addition to Yellow Bottleneck and Red Globe which are commonly grown in the Pleasant Valley district, 34 varieties of onions were tested in 1929, especially for their reaction to yellow dwarf infection but also for maturity and yield. [Henderson (7)]. The plot consisted of 198 1-rod rows, with alternate 3-row sections, planted with either Yellow Bottleneck or Red Globe onions for checks. Final disease readings were made June 20, and maturity and yield determined July 10 when the plot was harvested.

All onion varieties except the Riverside Sweet Spanish manifested yellow dwarf symptoms in the field tests of 1929. The varieties tested, in addition to Riverside Sweet Spanish, were: Yellow Flat Danvers, Yellow Globe Danvers, Vaughan's Prize-taker, Ohio Yellow Globe, Southport Yellow Globe, Vaughan's

Yellow Danvers, Yellow Dutch Vaughan, Vaughan's Special Yellow Globe, Phil. Yellow Dutch, Mountain Danvers, Michigan Yellow Globe, Giant Gibraltar, Vaughan's White Globe, Brown Australian Flat, Extra Early Flat Red, Vaughan's Red Globe, Bottle Onion, Bermuda Red, Extra Early Red Livingston, Reuther's Red Creole, Large Red Weathersfield, Red Weathersfield, Sutton's Lemon Rocca, Sutton's Red Rocca, Sutton's Ailsa Craig, Sutton's Imperial Reading, Mammoth Silver King, Crystal White Wax, Extra Early Barletta, Potato Onion, Yellow Bottleneck, Common Yellow Globe, Ebenezer, and Schutter Red Globe.

The visible infection found in the plot varied from 0 to 20 percent. Seemingly maturity time did not influence manifestation of yellow dwarf symptoms. The same strain of Riverside Sweet Spanish onions that was tested in 1929 was planted in a 1/100-acre plot April 21, 1930. The susceptible Red Globe variety was planted in an adjoining 1/100-acre plot as a check. These plots were adjacent to a field of Yellow Bottleneck table onions grown from sets, which showed 14 percent yellow dwarf. Plants in both plots were observed at weekly intervals and June 25, when the final readings were made, 10.0 percent of the Red Globe onion plants showed symptoms of yellow dwarf and none of the Riverside Sweet Spanish plants showed signs of infection. June 26 the bulbs from these plots were harvested and stored. Nov. 5, 1930, 200 Riverside Sweet Spanish bulbs and 100 Red Globe bulbs from the above plots were planted in the greenhouse to determine whether masking of symptoms had occurred.

TABLE 14. RESULTS OF INOCULATING RIVERSIDE SWEET SPANISH AND YELLOW BOTTLENECK ONION BULBS WITH YELLOW DWARF VIRUS TO SHOW THEIR RELATIVE RESISTANCE.

Exp. no.	Date inoculated	Number plants				Percentage infected plants			
		Virus inoculated		Checks		Virus inoculated		Checks	
		Riverside Sweet Spanish	Yellow bottle-neck	Riverside Sweet Spanish	Yellow bottle-neck	Riverside Sweet Spanish	Yellow bottle-neck	Riverside Sweet Spanish	Yellow bottle-neck
1	Oct. 10, 1931	25	25	20	20	No	80.0	No	No
2	Oct. 16, 1931	10	10	10	10	in-	70.0	in-	in-
3	Oct. 10, 1932	20	20	10	10	fec-	90.0	fec-	fec-
4	Dec. 20, 1932	20	20	15	15	tion	80.0	tion	tion
5	Jan. 30, 1933	20	20	15	15		90.0		
6	Feb. 12, 1933	20	20	15	15		60.0		
7	Mar. 1, 1933	20	20	15	15		60.0		
8	Apr. 1, 1933	20	20	15	15		50.0		

Fifty healthy Yellow Bottleneck onions, a susceptible variety, were planted as a check on the greenhouse conditions. When final readings of the greenhouse test were made Nov. 5, 1930, 12 percent of the Red Globe plants showed yellow dwarf symptoms, while none of the Riverside Sweet Spanish showed any signs of the disease. The Yellow Bottleneck onions planted as a check to greenhouse conditions showed no disease.

Results obtained in the field tests of 1929 and 1930, indicated that the Riverside Sweet Spanish strain was either highly tolerant of the yellow dwarf virus or it was not desirable as a host for the vectors that were transmitting the virus in the field.

ARTIFICIAL INOCULATION OF RIVERSIDE SWEET SPANISH ONIONS

Riverside Sweet Spanish was the only onion variety in the 1929 and 1930 field experiments that was not visibly infected with yellow dwarf virus under conditions in which 10 to 20 percent of the plants of all the other varieties were infected. Immediately this variety became important as a possible means of controlling the disease. To determine whether absence of the disease in this variety was due to escape or resistance, eight artificial inoculation experiments were conducted in the greenhouse during 1931 to 1933. A total of 155 Riverside Sweet Spanish onion bulbs was hypodermically inoculated in the meristematic tissue with juice from yellow dwarf infected onion leaves. As check on the in-



Fig. 11. Greenhouse indexing of onion bulbs for yellow dwarf disease (14 days after planting).



Fig. 12. Greenhouse indexing of onion bulbs for yellow dwarf disease. Same plants as shown in Fig. 11, 10 days later.

fectivity of the virus, an equal number of Yellow Bottleneck onion bulbs was treated similarly at the same time. Also, as further checks, onion bulbs of both varieties were injected with healthy onion juice. The bulbs were planted in 8-inch pots of steamed soil and held in a greenhouse separated from other onion plants. Readings were made 20-30 days after inoculation; the data are recorded in table 14.

In every experiment shown in table 14, the Riverside Sweet Spanish onion plants which were inoculated in the bulb with yellow dwarf virus, failed to show signs of the disease. The Yellow Bottleneck onion plants inoculated in a like manner with virus from the same samples of onion juice used for the Riverside Sweet Spanish inoculations were 50 to 90 percent visibly infected. Riverside Sweet Spanish and Yellow Bottleneck onion plants inoculated with healthy onion juice and, therefore, used as checks, showed no signs of the disease.

As a further test, healthy bottleneck onion bulbs were hypodermically inoculated in the meristematic tissue with juice extracted from leaves of yellow dwarf inoculated Riverside Sweet Spanish plants which were used in experiments 5, 6, and 7, table 20. None of the plants manifested yellow dwarf symptoms, showing that the Riverside Sweet Spanish plants that were inoculated with yellow dwarf virus were healthy and not masking yellow dwarf symptoms.

The strain of Riverside Sweet Spanish onions used in these experiments is somewhat later in maturing than is desirable for the Pleasant Valley district. Selections for earliness are being made.

During the course of experimental work in the greenhouse and field on varietal response of onions to yellow dwarf infection, it was found that the Riverside Sweet Spanish strain, in general, was visited by fewer numbers of onion thrips (*Thrips tabaci* Lind.) than the other varieties. Even when large numbers of thrips were present on this strain of onions, the plants were not damaged to the extent of causing white blast as was found in the other varieties tested.

INDEXING ONION BULBS FOR YELLOW DWARF INFECTION

In an earlier part of this paper it was shown that yellow dwarf virus overwintered in onion bulbs. It was also stated that more than 75 percent of the table onion crop in the district was grown from sets and that practically all of the infected seedlings for sets and a high percentage of the infected plants of the table onion crop masked yellow dwarf symptoms. Because of masking, selection of apparently healthy onions from this district for planting purposes gave practically no benefit.

A method was devised, therefore, whereby sample lots of onion sets and mother bulbs, collected at random from each grower's planting stock, could be indexed for yellow dwarf. [Henderson (6)]. The method of indexing was that of re-growing sample lots of sets and mother bulbs in greenhouse beds or in water cultures under conditions whereby the percentage of infected bulbs in each lot could be determined. The results of the indexing tests were given to the growers, together with recommendations to destroy the planting stocks that showed infection. This procedure seemed to eliminate the most important source of inoculum in the disease area.

Indexing tests were conducted for the years 1928 to 1933 inclusive and are summarized below:

TABLE 15. PERCENTAGE OF YELLOW DWARF INFECTED PLANTS IN THE PLEASANT VALLEY ONION DISTRICT FROM 1928 TO 1934, INCLUSIVE, AS SHOWN BY ANNUAL INDEXING RESULTS AND FIELD COUNTS.

Onion set crop	Number of growers	Onion sets indexed in greenhouse				Table onion crop for the district			
		Total number	Number healthy	Number diseased	Percentage infected	Season	Number plants inspected	Number plants infected	Percentage infection
1926	—*	—	—	—	—	1927	—**	—	1.0
1927	—	—	—	—	—	1928	100,000	40,000	40.0
1928	71	4,424	3,540	884	19.0	1929	98,000	22,850	23.0
1929	30	5,800	5,431	369	6.0	1930	75,000	6,010	8.0
1930	29	6,089	5,732	357	6.0	1931	75,000	4,670	6.0
1931	73	11,019	10,985	34	0.3	1932	75,000	387	0.5
1932	93	9,340	9,640	13	0.1	1933	—**	—	Trace
1933	71	7,675	7,673	2	0.03	1934	—	—	Trace

*No sets indexed in greenhouse until 1928.

**Ocular estimation.



Fig. 13. Production of Yellow Bottleneck onion sets in an isolated disease-free area for planting stock.

1928 INDEXING TESTS

Oct. 12, 71 sample lots, 60 to 90 onion sets each, and sample lots of 12 to 60 mother bulbs each, were planted in greenhouse beds. At the same time 200 healthy onion sets, grown outside the disease area, were planted as checks on the possible natural transmission in the greenhouse. Readings of the percentage of infection were made 16-20 days after planting. Figures 11 and 12 show the method of indexing onions by growing them in the greenhouse.

1929 INDEXING TESTS

In the spring of 1929, 11 to 315 onion sets from the same lots that were indexed in the greenhouse were indexed in field plots at Pleasant Valley, as a check on the results of the greenhouse indexing. Two hundred healthy onion sets were planted as an indicator of natural transmission of the yellow dwarf virus. As a further check on the results obtained by the greenhouse indexing tests, the percentage of infected plants in table onion crops grown from the different stocks of sets which were indexed in the greenhouse was determined. This was done by inspecting 2,000 plants in groups of 100 throughout each of the fields.

In 1929, 1930, 1931, 1932, and 1933, 50 to 400 onion sets and 8 to 90 mother-bulbs from each of the (respective) sample lots collected each year were indexed in the greenhouse. The results are given in table 14.

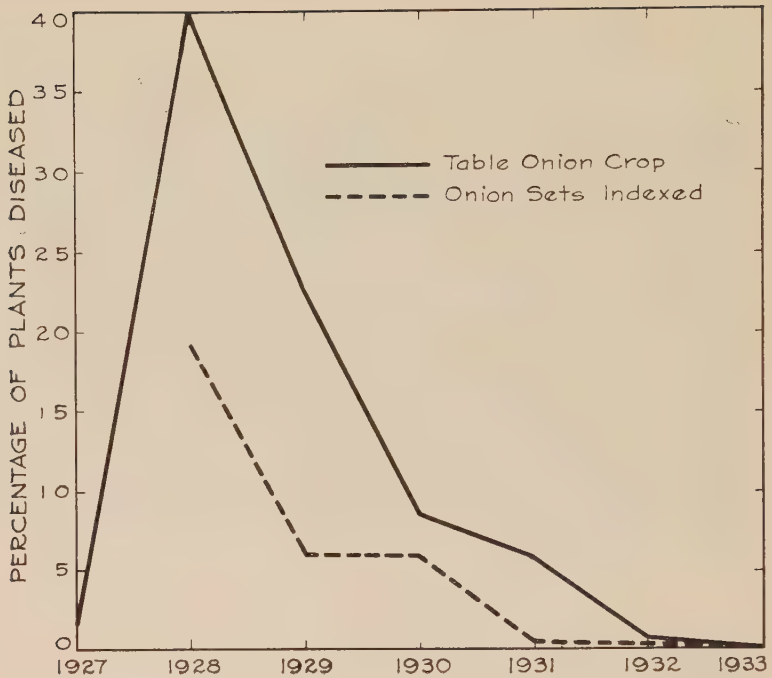


Fig. 14. Percentage of visible infection found in the indexing tests and table onion crops.

As shown in table 15, the percentages of yellow dwarf infection occurring in the 1928 sample lots indexed in the greenhouse and field were approximately the same as was found in the table onion crops grown from the respective stock of sets. These results show that indexing sample lots of planting stock gave the growers reliable estimates of the percentage of yellow dwarf infected onion bulbs in their planting stocks.

WATER CULTURE METHOD OF INDEXING ONIONS

In an effort to find a method of indexing whereby large numbers of bulbs could be grown in a small space, a number of trials were made in which sets and mother bulbs were grown in large pans of water. They were held in place in the water by resting on hardware cloth which was adjusted so that only the bulb plates were immersed. Smaller mesh was used for sets than for mother bulbs. Indexing the bulbs by this method gave accurate results when compared to the greenhouse bed method of indexing. Not only did this method save greenhouse space when large numbers of bulbs were indexed, but was one which could be employed by placing the pan near a

window in the home where there was sufficient light for rapid growth.

The role of indexing in controlling yellow dwarf will be discussed later along with other methods.

PRODUCTION OF ONION BULBS FOR PLANTING PURPOSES IN DISEASE-FREE AREAS

In 1928 it was noted that table onion crops from sets produced in areas where yellow dwarf infection was prevalent, showed much larger percentages of visibly infected plants than crops from sets grown in areas where the infection was absent or scarce. The main source of virus for infecting healthy plants of the current season crop was in the plants, which developed early from infected bulbs. When healthy—rather than yellow dwarf infected—bulbs were planted, the early seasonal source of infection was eliminated. Therefore, in addition to having all planting stocks of onion bulbs indexed, the growers were advised to produce their planting stocks of sets and mother bulbs in areas free from the yellow dwarf disease (fig. 13). In addition, they were advised to destroy the infected volunteer onion plants in the fields, because it had been found that they often overwintered the yellow dwarf virus.



Fig. 15. A crop of Yellow Bottleneck onions on the Gimm farm, Pleasant Valley, Iowa, 1930. The onions were grown from sets produced in the disease area and were 95 percent diseased at this time.



Fig. 16. A crop of Yellow Bottleneck onions on the Heeney farm, Pleasant Valley, Iowa, 1930. The onions were grown from sets that had been produced in a disease-free area and showed no evidence of yellow dwarf.

RESULTS OBTAINED FROM CONTROL MEASURES

Yellow dwarf was controlled by (a) indexing, (b) producing healthy planting stocks of bulbs in yellow dwarf-free areas and (c) roguing out infected volunteer onion plants.

The percentage of infection found in the total number of onion sets indexed in the greenhouse for each of the years 1928-1933, inclusive, and the percentage of infected plants in the entire district, which was based on field counts each year from 1928-1932, inclusive, shows a decline in prevalency of yellow dwarf infection. This is doubtless because of the control measures employed. These results are given in table 21 and fig. 14.

The percentage of yellow dwarf found in the district for each of the seasons 1928-1934, inclusive, was approximately that shown by the indexed sets planted. As a result of the adopted control measures, yellow dwarf infection declined from 40 percent in 1928 to a trace of the disease in 1934, as shown in fig. 14. According to R. M. Rice, president of the Pleasant Valley Onion Growers' Association, very few infected plants were found within the district during the season of 1934.

Yellow dwarf persisted longer, no doubt, because growers failed in many instances, to destroy stocks of onion sets and

mother bulbs which were shown by indexing to be infected, and because a few growers produced sets locally.

In one instance in 1929, locally grown sets and mother bulbs were planted in 1930 without being indexed. The table onion crop produced from the locally grown sets was 95 percent visibly infected, fig. 15, while a table onion crop grown from sets produced in a yellow dwarf free area and indexed were healthy, fig. 16. The diseased crop yielded 35 bushels per acre, fig. 17, while a healthy table onion grown from disease-free sets (produced in an area free from disease and which were indexed) yielded 900 bushels per acre, fig. 18. The plants grown from infected mother bulbs selected from locally grown crops were 85 percent visibly infected, fig. 19, while a similar seed crop from healthy mother bulbs, fig. 20, which was produced in an area free from yellow dwarf and indexed was healthy. Those growers who produced their planting stock in yellow dwarf-free areas and otherwise practiced the recommended control measures had very little yellow dwarf disease in their crops.

The one instance cited was somewhat unusual, as other growers who raised sets locally in 1929, produced crops having only 12 percent visible infection. These growers, however, by such practices, were responsible for the presence of early seasonal infection and a source of inoculum.

After 1930 all growers in the district practiced the recommended control measures, namely, indexing all planting stocks of bulbs, producing all planting stocks of bulbs in areas free from yellow dwarf infection, and roguing out infected volun-



Fig. 17. A crop of Yellow Bottleneck onions on the Gimn farm which were 95 percent diseased and yielded 35 bushels per acre in 1930. The crop was grown from sets produced within the disease area.



Fig. 18. A crop of Yellow Bottleneck onions grown from healthy sets on the F. H. Schutter farm, Pleasant Valley, Iowa, yielding 900 bushels per acre in 1930.

teer onion plants. Evidence of the effect of these control measures in the control of yellow dwarf infection in the district is indicated by a decline from 40 percent in 1928 to a trace of infection in 1934.

The study of the yellow dwarf disease of onions in Iowa has evolved practical control measures adequate to protect the industry against this menace; carelessness in allowing small amounts of the disease to persist may, at any time, cause a recurrence of yellow dwarf in epiphytotic form.



Fig. 19. Red Globe onion plants, 85 percent diseased, on the Gimm farm, 1930. The crop was grown from mother-bulbs produced within the disease area.



Fig. 20. Red Globe onion plants with no yellow dwarf present, on the Schutter farm, 1930. The crop was grown from mother bulbs produced outside the disease area.

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